Journal of the Serbian Chemical Society
JSCS-info@shd.org.rs • www.shd.org.rs/JSCS

J. Serb. Chem. Soc. *82* (6) 723–737 (2017)
JSCS–4999

UDC 504.054:546.27'42'47:581.43/.45:
582.632.1:581.132(497.11)
Original scientific paper

Possibilities of assessing trace metal pollution using *Betula pendula* Roth. leaf and bark – Experience in Serbia

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(Received 13 January, revised 14 February, accepted 17 February 2017)

Abstract: In this study, both seasonal and spatial variations in trace metal uptake, as well as concentration of photosynthetic pigments in Silver birch (*Betula pendula* Roth.) trees in five urban parks in Pančevo, Smederevo, Obrenovac and Belgrade (Serbia) affected by different anthropogenic activities were studied. The characteristics of soil were assessed in terms of texture, pH and trace element content. Concentrations of boron, strontium and zinc in both leaves and bark showed an increasing temporal trend, however, copper showed an opposite seasonal trend. A higher accumulation of trace elements was noticed in leaves compared to bark. The obtained results for photosynthetic pigments showed low sensitivity of birch to B, Cu, Sr and Zn contamination, indicating that birch tolerates pollution and climate stress by increasing the amount of pigments. Analysis of the effects on soil chemistry of trace element accumulation in plant tissues proved that soil chemistry poorly explains the variability of elements in bark (27.6 %) compared to leaves (82.99 %). Discriminant analysis showed that Belgrade and Smederevo are clearly separated from the other three sites.

Keywords: urban pollution; biomonitoring; photosynthetic pigments; plant tissue; soil.

INTRODUCTION

Increasing industrialization and urbanization coupled with increased vehicular traffic intensifies the emission of various pollutants, such as trace elements, into the atmosphere.[1] The urban environment is exposed to anthropogenic contaminants released from both stationary (power plants, industries and waste disposal) and mobile (road traffic) sources. Trace elements are persistent and widely

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https://doi.org/10.2298/JSC170113024P

dispersed in the environment and can interact with different natural components, which results in toxic effects on the biosphere.[2] Heavy metals induce morphological, biochemical and physiological changes in plants.[3,4]

Trees, as one of the most abundant plant types together with grass, found in urban green areas, intercept atmospheric pollutants from the air (through wet and dry deposition) and accumulate metals from the soil by their root system that later translocate to other plant parts.[2,5] Trace element absorption from soil is governed by soil characteristics such as the total concentration of elements present in the soil, pH, clay and hydrous oxide content, organic matter and redox conditions.[6] Many studies have shown that higher plants, especially different parts of trees (roots, leaves and bark), can be successfully used as biomonitors due to their accumulation properties, availability of biological material, simplicity of species identification, sampling and treatment, as well as their good tolerance to environmental changes.[5–10] Leaves are a often used for monitoring elemental deposition from the atmosphere. The deposition of pollutants on the leaf surface can be affected by the morphological and structural properties of the leaf, such as orientation and size, cuticle thickness, roughness, existence of surface wax layer.[8] Likewise, tree bark as a non-living plant material is also considered to be a promising indicator in air pollution monitoring and its surface has a very important influence on the accumulation of pollutants due to its structural porosity. Furthermore, factors such as the concentrations of pollutants in the air, physiological and chemical properties of the bark, through fall, soil factors, contamination from other plants, climatic factors, *etc.*, can also affect element accumulation.[7,8]

High accumulation of metals and other pollutants in plant photosynthesizing tissues can cause changes in the concentration of photosynthetic pigments (chlorophyll and carotenoids), which directly affects the plant productivity. Therefore, their levels are an important tool used for the evaluation of the effects of air pollutants on plants.[1,11]

Silver birch (*Betula pendula* Roth.) was studied in five urban parks in the localities exposed to different sources of pollution: Pančevo, Smederevo, Obrenovac, Belgrade and the *Arboretum* of the Faculty of Forestry in Belgrade (which is unexposed to a direct source of pollution). The elements B, Cu, Sr and Zn were selected to be analyzed because B is one of the essential micronutrients for plants, Cu is involved in many physiological processes in plants, Sr is toxic and shares some chemical and physical characteristics with Ca, therefore can enter the food chain and alter physiological process and Zn is an essential element with a fundamental role in plant metabolism. Seasonal variations in trace element concentrations (B, Cu, Sr and Zn) were analysed in leaves and bark, as well as the content of photosynthetic pigments (Chlorophill a and b, Chl a and Chl b, and total carotenoids, Tot Carrot) in leaves, in order to assess the impact of urban environment pollution on the health status of *B. pendula*. In addition, the texture, pH and trace element concentration in soil was determined in order to distinguish the elements' mobility and availability. Silver birch has shallow roots spreading around the tree in all directions, so the sampled soil was always tightly bound to birch roots. The bioconcentration factor was determined to further clarify whether birch is a potential accumulator, indicator or excluder of trace elements.[12,13]

Previous studies provided evidence that *B. pendula* is good bioaccumulator of trace elements in leaves, especially for Zn, and is suitable as a biomonitor of trace element contamination due to its resistance to different types of industrial pollution.[9,14-16]

The aim of this study was to: 1) discern between significant variations throughout vegetation season in B, Cu, Sr, and Zn content and their potentially toxic concentrations in leaf, bark and soil of *B. pendula*; 2) to investigate the effects of industrial and traffic pollution on the chlorophyll level in birch trees; 3) to compare the accumulation levels between bark and leaf samples of *B. pendula*.

EXPERIMENTAL

Soil and plant samples analyses

Plant and soil samples were collected from five urban parks in the localities exposed to different sources of pollution: Pančevo, Smederevo, Obrenovac and Belgrade. Detailed information on study area, species description and sampling are given in Supplementary material to this paper.

Soil pH values were measured using a glass electrode (1:2.5 soil-water ratio) after shaking the samples to equilibrium for approximately 30 min.[17] The pH was measured directly in the suspension.

Leaves, bark and soil were dried to constant weight at 105 °C (Binder, Tuttlingen, Germany). Dried samples were ground using a stainless-steel mill and sieved through a 1.5 mm stainless-steel sieve. For trace element analysis, the leaf, bark and soil samples (0.3 g) were digested in a microwave (CEM, 39 MDS-2000), using the method USEPA 3052. The final extracts were filtered into 50 mL polyethylene volumetric flasks, and then diluted to the mark with deionized water.

The concentrations of B, Cu, Sr and Zn in the samples were measured by inductively coupled plasma optical emission spectrometry (ICP-OES) (Spectro Genesis Fee, Spectro-Analytical Instruments GmbH, Kleve, Germany). Quality control was performed using the certified reference material for leaves–Beech leaves–BCR–100, and soil–Loam soil–ERM–CC141 obtained from the IRMM (Institute for Reference Materials and Measurements, Geel, Belgium) and is certified by the EC-JRC, European Commission−Joint Research Center.

The concentrations found were within 95-110 % of the certified values for all measured elements. Element concentrations were expressed as mg per kilogram of the dry leaf weight (mg kg^{-1} d.w.).

Chlorophyll concentration in the leaf tissue was determined spectrophotometrically based on the light absorption of a solution obtained after extraction with dimethyl sulfoxide (DMSO).[18] One disk (1 cm in diameter) per leaf was harvested from 5 leaves. Chlorophylls and total carotenoids were extracted with 1 mL of DMSO. After incubation at 65 °C until full extraction of chlorophyll was reached, the absorbance of extracts was measured at 663, 645 and 480 nm with UV–visible spectrophotometry (Shimdazy UV–160). The equations of Arnon[19] were used to calculate Chl a and Chl b. Total carotenoids were calculated according to Wellburn.[20] Chlorophyll and carotenoid concentrations in leaves were expressed also in mg per gram of the dry leaf weight (mg g^{-1} d.w.).

Calculation of bioconcentration factor (BCF)

Plant-to-soil bioconcentration (BCF) ratio estimates for element accumulation ability of the plants from the soil[21] was calculated as a ratio of metal content in plant part (leaf and bark) and substrate expressed on a dry-weight basis.

Statistical analyses

The results were analyzed using statistical analysis (ANOVA) by computing the statistical significance of the mean differences of photopigments (Chl a, Chl b, Tot Carot) and trace element concentrations with respect to their locations.

Linear Discriminant Analysis[22] was performed to assess the differentiation among the analyzed sites based on the variations of Chl a, Chl b, Tot Carot and elements.

Relationships among concentrations of all elements in the soil and tissues of *B. pendula* were assessed using both canonical correlation analysis (CCA)[23] and Mantel's test[24-26]

In order to assess the effects of soil chemistry on the concentrations of elements in birch tissues, redundancy analysis[17] was performed. Statistical analyses were performed using the "FLORA" software package.[28]

RESULTS AND DISCUSSION

Trace element content in soil and plant material

In the examined soil samples, the pH in aqueous solutions ranged from 8.30 at the control site to 8.51 in Smederevo (Table S-I of the Supplementary material), which puts these soils into a group of alkaline soils.[29] Based on a specifically constructed triangle for soil classification by texture in parks in Pančevo, Smederevo, Belgrade and at the control site, the dominant soil type was sandy clay loam, while in the park in Obrenovac clay loam was ascertained.[29]

The concentrations of the examined elements in the soil were analyzed in order to characterize potential soil pollution and to determine the transfer efficiency from the soil. The seasonal variation in B, Cu, Sr and Zn in soil, leaves and bark of *B. pendula* from each sampling site are provided in Tables I and S-I.

Boron is one of the essential micronutrients for plants. It is unique among other essential elements in that a narrow range in its concentration can mean the difference between plant deficiency and plant toxicity.[30] In our study, the average concentration of B in the soil from the five examined sites ranged from 128.63 mg kg^{-1} in Pančevo to 166.28 mg kg^{-1} at the control site, and all obtained values were above the normal range for soil, as described by Kabata-Pendias and Pendias,[31] Table S-II.

Seasonal fluctuations of B concentrations in leaves were noted. The lowest concentrations of B were measured in June in Obrenovac (30.96 mg kg^{-1}), and the highest in October at all sites; toxic concentrations (Table S-II), as defined by Kabata-Pendias and Pendias,[31] were measured in Belgrade (276.88 mg kg^{-1}). Toxic

B concentrations in soil can influence the B bioavailability for plants, which is linked to soil pH.[32] Namely, the availability of B to plants is highly dependent on pH, with a maximum at pH > 7.[31] Also, under conditions of low rainfall, as was the case throughout the entire year of the study (2012), B was not being sufficiently leached from soil surface and could therefore accumulate to toxic concentrations.[33] The apparent accumulation of B to a toxic concentration resulted in the appearance of toxicity symptoms on older leaves in the form of marginal or tip chlorosis, or both, and necrotic patches.

TABLE I. Average trace element concentrations (mg kg^{-1} d.w.) in leaves and bark of *B. pendula* sampled from urban sites Pančevo-P, Smederevo-S, Obrenovac-O, Belgrade-B, control-C. All values are mean with the standard deviation in parentheses (n = 5)

Site	June				August				October			
	B	Cu	Sr	Zn	B	Cu	Sr	Zn	B	Cu	Sr	Zn
						Leaf						
P	36.59	4.89	34.42	29.80	44.53	4.08	36.28	24.88	59.20	3.63	21.61	39.39
	(0.12)	(0.15)	(0.29)	(0.68)	(0.51)	(0.26)	(0.28)	(0.63)	(0.65)	(0.13)	(0.17)	(0.30)
S	106.29	3.67	36.19	20.92	134.57	3.75	44.04	51.29	191.39	4.22	34.93	63.67
	(0.65)	(0.18)	(0.45)	(0.51)	(1.22)	(0.14)	(0.41)	(0.73)	(1.53)	(0.21)	(0.41)	(0.67)
O	30.96	5.81	35.55	36.68	79.00	3.72	41.99	38.29	86.11	4.10	29.73	34.36
	(0.62)	(0.49)	(0.27)	(0.68)	(0.75)	(0.27)	(0.51)	(0.68)	(0.85)	(0.21)	(0.59)	(0.29)
B	168.82	5.86	51.57	199.54	269.14	3.94	48.50	332.07	276.88	4.77	46.15	344.96
	(2.74)	(0.23)	(0.19)	(1.06)	(2.94)	(0.10)	(0.28)	(4.60)	(1.65)	(0.08)	(0.37)	(2.10)
C	45.89	5.62	28.98	30.02	67.80	4.64	31.44	40.41	105.56	4.03	27.14	35.52
	(1.52)	(0.14)	(0.64)	(0.65)	(0.47)	(0.09)	(0.21)	(0.52)	(0.72)	(0.12)	(0.74)	(0.27)
						Bark						
P	1.20	5.55	10.03	72.04	4.64	5.65	5.58	135.37	2.98	5.00	3.26	162.77
	(0.08)	(0.19)	(0.18)	(0.68)	(0.15)	(0.30)	(0.16)	(0.71)	(0.24)	(0.08)	(0.15)	(1.02)
S	5.01	5.58	13.17	73.34	7.81	5.19	7.93	145.41	16.63	5.80	9.12	162.69
	(0.28)	(0.13)	(0.62)	(1.12)	(0.30)	(0.41)	(0.35)	(3.45)	(0.55)	(0.04)	(0.36)	(2.27)
O	4.11	4.15	13.16	53.69	9.42	4.50	10.43	85.88	5.60	3.83	3.53	127.02
	(0.12)	(0.08)	(0.12)	(0.64)	(0.34)	(0.18)	(0.33)	(1.25)	(0.20)	(0.11)	(0.37)	(12.79)
B	9.02	9.25	21.26	74.41	14.53	12.49	18.24	105.06	11.96	4.72	9.89	160.15
	(0.20)	(0.37)	(0.43)	(0.54)	(0.14)	(0.29)	(0.45)	(0.37)	(0.34)	(0.16)	(0.26)	(4.50)
C	4.55	5.95	12.27	45.78	5.64	5.95	6.94	116.03	3.50	4.24	4.29	120.59
	(0.23)	(0.18)	(0.35)	(0.82)	(0.24)	(0.17)	(0.18)	(0.02)	(0.34)	(0.44)	(0.28)	(0.75)

Unlike leaves, B concentrations in tree bark were very low during the growing season (Table I). The lowest concentration was measured in Pančevo in June (1.20 mg kg^{-1}) and the highest at the end of season in Smederevo (16.63 mg kg^{-1}).

Copper is an essential transition metal that is involved in many physiological processes in plants.[35] The concentrations of Cu in the soil at all five investigated sites were in the normal range for soils (Table S-II). The lowest concentration was measured in Pančevo (11.93 mg kg^{-1}). The highest concentration was determined in Smederevo (30.66 mg kg^{-1}).

The highest Cu content at all examined sites was observed at the beginning of the vegetation season, with the highest (5.86 mg kg^{-1}) measured in Belgrade. This spike was followed by a decrease in the second part of the season. These results indicate a higher rate of plant metabolic activity in the first part of the season, resulting in a stronger uptake rate and metal accumulation. A similar seasonal trend was previously observed by Kim and Ferguson[36] and to some extent by Piczak *et al.*,[15] who noted that Cu concentrations were highest in new leaves and gradually decreased with time. In our study, in the second part of the season Cu levels were below values for normal plant growth, and symptoms of deficiency occurred in the form of leaf-tip chlorosis or necrosis in young leaves, which later spread downward along the leaf margins in older leaves. In general, the deficiency effects of Cu on plants are usually noted at concentrations lower than 5 mg kg^{-1}. Copper-deficient plants show morphological changes in leaf architecture and this represents the first symptom of Cu deficiency.[37]

Notwithstanding the normal concentration of copper in the soil (Table S-II), it is likely that the alkaline conditions (pH > 8.0) and Cu low transfer coefficients cause lower element solubility and lower availability for plants, which explains its deficit in leaves.[38,39] The concentration of Cu in bark remained relatively constant from June to October, which is consistent with the findings of Reimann *et al.*[40] on the seasonal dynamics of different elements in birch leaves and bark under different anthropogenic conditions, *i.e.*, Cu values in the leaves and bark remained almost the same during the examined season. The only exception was Belgrade with a maximum in August (12.49 mg kg^{-1}) and a minimum in October (4.72 mg kg^{-1}), which could be explained by the abundance of roads and traffic emissions, since most of the Cu in urban environment originates from traffic and use of brakes.[41]

Strontium is a toxic, nonessential element to plants which shares some chemical and physical characteristics with calcium, which is an essential element required for plant growth. The content of strontium in plants is highly variable.[42] In our study, the concentration of Sr in the soil exhibited considerable variability, ranging from a minimum of 18.32 mg kg^{-1} measured in Pančevo, to a maximum of 85.53 mg kg^{-1} in Obrenovac. However, all the measured values were below the average concentration for soils on a world scale (Table S-II).

The concentration of Sr in plants is very variable, although the highest concentrations are usually observed in plant tops.[31] In this study, toxic concentrations of Sr in birch leaves (>30 mg kg^{-1})[34] were measured throughout the season at all the examined localities. The maximum concentration were reached during August, except in Belgrade where it was reached in June (51.57 mg kg^{-1}), while the lowest during October at all sites. Given the fact that concentrations in range[31] of 1–10 mg kg^{-1} are considered as normal Sr values for plant tissues, concentrations in range of 21.61–29.73 mg kg^{-1} obtained for Pančevo, Obrenovac and control site during October could be interpreted as excessive or even toxic. Piczak *et al.*[15] observed an increase in Sr content in birch leaves with time. In general, leaves have the strongest accumulation capability among plant tissues.[42]

Compared to leaves, the concentrations in the bark were much lower (in the range of 5–21 mg kg^{-1}, Table I), which is in accordance with previously published results regarding Sr content in birch leaf and bark.[40] The highest concentration of Sr in the bark, at all five analyzed sites, was measured in June (maximum in Belgrade, 21.26 mg kg^{-1}), followed by a decrease through the season, with lowest values measured in October (minimum in Pančevo, 3.26 mg kg^{-1}). Also, Sr follows a similar distribution in plant tissues as observed for B; however, the relative accumulation of Sr in leaves is not as extreme.

After comparing the annual average concentration of Sr in leaves and soil, it was observed that the concentration of Sr in soil is slightly higher or equal to its concentration in leaves. Bearing in mind that the uptake of Sr by plant roots depends on the plant species and soil properties, such as organic matter content, pH and ionic composition,[6] and that the root uptake of Sr is much greater than leaf uptake,[42] it can be assumed that a part of Sr contents is airborne and deposited on leaf surfaces.

In our study, concentrations of Zn in soils were within the normal level for soils worldwide (Table S-II). The highest Zn content was measured in Belgrade (134.53 mg kg^{-1}).

For plants, Zn is an essential element with a fundamental role in metabolism.[31] Unlike copper, the Zn content in leaves was the lowest at the beginning of season, with the lowest concentration (20.92 mg kg^{-1}) recorded in Smederevo, reaching the maximum in late season. These results are in accordance with the findings of Piczak *et al.*[15] Likewise, Kabata-Pendias and Mukherjee[31] found that Zn is predominantly concentrated in mature leaves or during the phase of intensive growth of plant. In our study, the Zn content was within the normal range during the entire season at all examined sites, except Belgrade. Toxic effects of Zn are observed at concentrations higher than 100 mg kg^{-1} (Table III). The concentrations recorded in Belgrade that ranged from 199.54 in June to 344.96 mg kg^{-1} in October were all within the toxic range and well over the values considered as optimal for plant development. Such high concentrations of Zn could be related to traffic. Abrasion of tires is a source of Zn contaminants in the environment, since Zn is a catalyst in the manufacture of tires and additives containing Zn are often used in tires and motor oil.[44] The phytotoxicity of Zn mostly depends on the species of plant, age, environmental conditions and the combination of other heavy metals.[45] Copper and zinc show antagonistic interactions, *i.e.*, the uptake of one element is inhibited by the other due to competition for the same sites for absorption into the plant root.[46] An excess of Zn can also give rise to Cu deficiencies in plant shoots.[47]

Zinc content in bark also showed an increasing tendency during the vegetation season with the lowest concentrations measured in June at the control site (45.78 mg kg^{-1}), and highest in October at all the sites, the highest levels being measured in Pančevo (162.77 mg kg^{-1}). Bark contained much higher Zn concentration than leaves, with the exception of the Belgrade urban park. These results agree with the findings of Reimann *et al.*[39] who found that bark was susceptible to a greater impact of urban pollution than leaves due to prolonged exposure time. High bioaccumulation of Zn in tree bark could be the consequence of its high transfer coefficient, which is a reflection of its relatively poor sorption in the soil.[37]

Bioconcentration factor

After determining the concentrations of B, Cu, Sr and Zn in soil, leaf and bark, their bioconcentration factor (*BCF*) was calculated to assess the possible role of *B. pendula* as a potential accumulator, indicator or excluder of these elements. *BCF* in *B. pendula* expresses the ratio of element concentrations (mg kg^{-1}) in plant parts (leaves and bark) to its concentrations in soil. A $BCF > 1$ indicates that the plant is enriched with metals (accumulator); $BCF = 1$ indicates relatively indifferent response of the plant to metals (indicator); $BCF < 1$ shows that the plant excludes metals from uptake (excluder).[12,13] The results in Table II shows that the *BCF* values for Cu ranged from 0.1–0.3, for B from 0.3–1.5 ($BCF > 1$ was obtained at Belgrade and Smederevo sites), for Sr from 0.4–1.6 ($BCF > 1$ was obtained for Pančevo and the control site), and for Zn they ranged from 0.3–2.1 ($BCF > 1$ was obtained for the Belgrade site). Considering that the uptake of trace metals from the soil affected by several factors (pH, organic matter content and soil type), it can be assumed that the characteristics of the soil, *e.g.*, clay loam in Obrenovac, is the main reason for the low accumulation of elements in birch leaves at this site. The *BCF* values for B in bark (Table II) were in the

TABLE II. Bioconcentration factor for *B. pendula* leaves and bark sampled from from urban sites. All values are mean with the standard deviation in parentheses ($n = 5$)

Site	Leaf				Bark			
	B	Cu	Sr	Zn	B	Cu	Sr	Zn
Pančevo	0.36 (0.01)	0.35 (0.01)	1.68 (0.04)	0.51 (0.03)	0.06 (0.03)	0.45 (0.01)	0.34 (0.01)	1.99 (0.11)
Smederevo	1.04 (0.02)	0.13 (0.01)	0.81 (0.02)	0.38 (0.01)	0.71 (0.01)	0.18 (0.01)	0.21 (0.01)	1.02 (0.01)
Obrenovac	0.42 (0.01)	0.30 (0.01)	0.42 (0.02)	0.81 (0.07)	0.40 (0.01)	0.28 (0.01)	0.11 (0.01)	1.96 (0.12)
Belgrade	1.57 (0.02)	0.21 (0.01)	0.97 (0.02)	2.17 (0.01)	0.08 (0.01)	0.38 (0.01)	0.33 (0.01)	0.84 (0.01)
Control	0.44 (0.01)	0.38 (0.01)	1.04 (0.05)	0.64 (0.01)	0.03 (0.01)	0.43 (0.01)	0.28 (0.02)	1.72 (0.01)

range from 0.03–0.7, for Cu from 0.2–0.4, for Sr from 0.1–0.3 and for Zn from 0.8–2.0 (*BCF* > 1 was obtained at all the examined sites except of Belgrade site).

Effects of soil chemistry on the concentrations of the analyzed elements in birch tissues

Nutrient-cycling processes mutually connect plants and soil. Plants absorb essential elements from soil. The absorption process ultimately depends on the availability of anions and cations in the soil. On the other hand, plants also control the nutrient-cycling process by nutrient-use efficiency, transpiration rate, root exudates, and by the tissue chemistry of falling leaves and twigs that are incorporated in decomposition processes. Despite the strong mutual relationship among plants and soil, the correspondence between soil and plant chemistry is relatively poor because the absorption of elements is a process controlled by the several factors, such as pH, organic matter content, redox conditions, *etc*.[6]

In order to detect mutual relations among the concentrations of all elements in the soil and tissues of *B. pendula,* we used canonical correlation analysis, CCA. A higher correlation coefficient was found between soil chemistry and the concentrations of B, Cu, Sr and Zn in leaves in comparison to bark (Table III). This indicates that bark chemistry is determined not only by soil chemistry, but also by other factors (*e.g.*, atmospheric deposits).

TABLE III. Hotelling's correlation among the concentrations of B, Cu, Sr and Zn in the soil and tissues of *B. pendula*

Principal axes	Soil and leaves of birch	Soil and bark of birch
1	0.853	0.243
2	0.311	0.044
3	0.194	0.034
4	0	0

The main disadvantage of canonical correlation analysis is the fact that the method calculates the correlation between two sets of variables for each principal axis. In order to detect the overall correlation between soil chemistry and the chemistry of birch tissues, Mantel's test was performed,[24,25] (Fig. 1). Mantel's test proved that the soil chemistry is significantly correlated with the concentrations of B, Cu, Sr and Zn in the leaves of *B. pendula.*

Biotic indicators require a causal relationship between biotic components and the environment. If the causal relation is perfect, then an ideal bioindicator unambiguously points to the environmental conditions of a site.[48] In order to detect causal effects of soil chemistry on the concentrations of the analyzed elements in birch tissues, we performed the redundancy analysis (RA). By the analogy with the univariate regression analysis, the redundancy analysis performs a variance partitioning to detect the effects of a set of independent variables on a set of dependent or "response" variables. Redundancy analysis suggests that soil chemistry poorly explains the variability of the elements in birch bark. Namely, soil chemistry explains only 27.6 % of variability of the analysed elements in the bark. However, the percentage of total variability of the elements in the leaves explained by soil chemistry is much greater (82.99 %), Table IV.

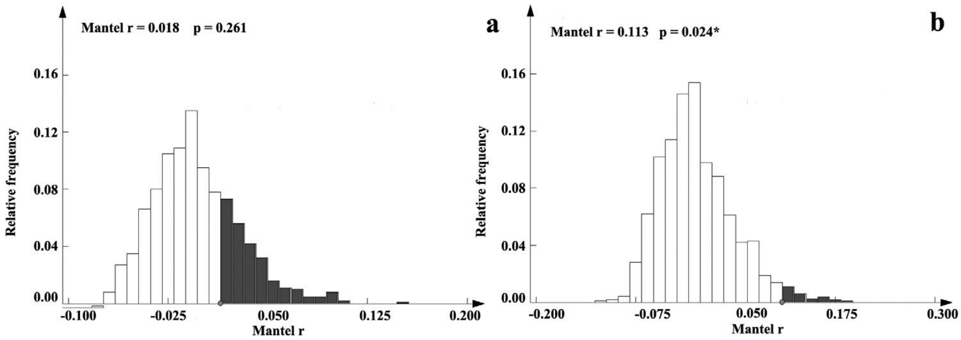

Fig. 1. Overall correlation between the chemistry of soil and birch bark (a) and soil and birch leaves (b).

TABLE IV. Causal effects of soil chemistry on concentrations of elements in leaf and bark of *B. pendula*

Variance of concentrations of elements in leaves of *B. pendula*	17418.3
Variance of concentrations of elements, predicted by regression model	14455.4
Variance explained by soil chemistry	0.8299
Variance of concentrations of elements in bark of *B. pendula*	1541.67
Variance of concentrations of elements, predicted by regression model	425.21
Variance explained by soil chemistry	0.276

Photosynthetic pigments

Chlorophylls and carotenoids are the main photosynthetic pigments in plants. Photosynthetic pigments have been used as sensitive markers of stress caused by heavy metals and other pollutants since they are very sensitive to changes in the environment. Therefore, their quantification provides useful insight into the physiological performance of plants. Carotenoids are accessory pigments and essential structural components of the photosynthetic antenna and reaction centers in higher plants. As non-enzymatic antioxidants, their main function is to protect the photosynthetic apparatus, dissipating energy to avoid harmful photooxidative processes.[11]

In our study, the concentration of Chl a ranged from 2.26 in Smederevo, to 6.82 mg g^{-1} in Obrenovac, and increased significantly in the second part of season (Table V). The lowest content of Chl a was measured in Belgrade during the entire vegetative season, which could be attributed to the toxic concentrations

TABLE V. Average Chl a, Chl b and Tot Carot (mg g^{-1} d.w.) in *B.pendula* sampled from urban sites in June, August and October 2012. The values are mean, with standard deviation in parentheses (*n* = 5)

Site	June			August			October		
	Chl a	Chl b	Tot carot	Chl a	Chl b	Tot carot	Chl a	Chl b	Tot carot
Pančevo	3.63	1.81	1.03	4.62	1.37	1.41	5.48	2.04	1.72
	(0.24)	(0.20)	(0.09)	(0.27)	(0.22)	(0.07)	(0.30)	(0.21)	(0.21)
Smederevo	2.26	2.50	0.93	3.44	1.27	1.27	5.09	1.52	1.22
	(0.19)	(0.25)	(0.12)	(0.29)	(0.18)	(0.16)	(0.34)	(0.35)	(0.52)
Obrenovac	2.80	2.16	1.05	5.93	1.81	1.78	6.82	2.03	1.05
	(0.06)	(0.14)	(0.02)	(0.19)	(0.08)	(0.18)	(0.09)	(0.14)	(0.51)
Belgrade	2.57	1.46	0.86	3.62	1.03	1.12	2.86	0.71	0.86
	(0.05)	(0.14)	(0.09)	(0.23)	(0.16)	(0.12)	(0.20)	(0.08)	(0.06)
Control	2.75	2.57	0.87	5.56	1.63	1.58	4.72	1.21	1.16
	(0.12)	(0.32)	(0.11)	(0.21)	(0.18)	(0.12)	(0.30)	(0.04)	(0.14)

of Zn and Sr in birch leaves. Moyen and Roblin[6] showed that Sr probably blocks ion channels in the chloroplast envelope and decreases the content of Mg, which is a main component of chlorophyll. In contrast, the highest Chl a content was measured in leaves samples from Obrenovac. Our present results do not suggest clear reasons for such birch behaviour, so we can only assume that sensitivity of species to any element depends on concentration, exposure period, age and growth conditions. Although, many authors have shown that an increased content in polluting agent leads to chlorophyll inhibition[11,49–51] either through direct inhibition of several enzymatic steps or as a result of substitution of the central Mg ion,[52] several studies have shown that the exposure to heavy metals induces oxidative stress which is accompanied by an increase in the chlorophyll content.[52–54] For example, Kalaikandhan *et al.*[55] examined the effects of different Zn concentrations on the photosynthetic pigment content in *Sesuvium portulacastrum* leaves during 120 days and established that an increase in Chl a accompanied the increase in the concentration of Zn in the substrate from 100 to 300 mg kg^{-1}, with which our results are in good agreement. Likewise, Petrova[56] tested the pigment response to urban air pollution in birch leaves and found that the highest concentrations of chlorophyll were present in plants growing in environments with a medium level of urbanization and moderate exposure to road traffic pressure.

Chl b content in birch leaves was in range from 0.71 in Belgrade to 2.57 mg g^{-1} at the control site (Table V); Chl b exhibited an opposite trend and decreased during the season. A similar trend was found by Gajić *et al.*[4] at polluted sites (urban parks of Belgrade), where higher content of Chl b in *Ligustrum ovalifolium* Hassk. was measured in June than in October. The observations presented in this study are also consistent with the findings of Fargašova[49] who examined the aboveground parts of *Sinapis alba* L. seedlings, however, our results are not in agreement with the findings of Keser *et al.*[54] in *Lepidium sativum* L. Different effects of pollution on Chl b in relation to Chl a have been reported in many studies.[52,54] However, the different responses of Chl a and Chl b are not unexpected, since different types of pollution exert different effects on the pigment content, hence the response of a plant can be attributed to the interaction between various types of pollutants as well as abiotic factors (high temperature, drought, intense insolation, *etc*.).

The minimum carotenoid concentration was measured in samples from Belgrade (0.86 mg g^{-1}) and the maximum in samples from Obrenovac (1.78 mg g^{-1}). Their content was the highest in August when the climate conditions were the most unfavourable (Table V). Namely, the studied year was the warmest and the least rainy year (related period 1981–2010), with extremely high number of hot days and nights, since the beginning of their measurement in Serbia. Mean summer air temperature in Serbia was categorized as extremely warm, with 62 days of maximum daily temperatures higher than 30 °C, and 52 nights with minimum temperatures higher than 20 °C.[57] An increase in carotenoid concentration could be seen as a defence strategy to suppress above mentioned conditions, which has been shown with earlier studies.[58]

Differentiation among examined sites

The difference in the total content of B, Cu, Sr and Zn and photosynthetic pigments in leaves of *B. pendula* between the examined sites was determined by discriminant analysis (DA). Based on the first discriminant function (DC1) which is responsible for 97.36 % of differences, and the second discriminant function (DC2) which explains 2.64 % of differences, it can be noted that the Belgrade and Smederevo sites are clearly separated from the other three sites. Overlap exists in the case of Pančevo, Obrenovac and the control where they partially overlap (Fig. S-2a of the Supplementary material). The parameters that most contribute to the separation are B, Sr and Chl b on DC1 and B and Zn on DC2 (Fig. S-2b).

CONCLUSION

The result of this research provides an insight of the pollution state of four urban localities in Serbia, that are exposed to different sources of pollution using *Betula pendula* leaf and bark.

The concentrations of B, Sr and Zn in leaves reached maximal and sometimes toxic levels during the second part of the season, in August and October, unlike Cu whose concentration was deficient or close to being deficient for normal plant development. A toxic concentration of B was measured in Smederevo and Belgrade, possibly as the result of high concentrations of B in the soil and conditions favouring its transfer from the soil to the plant. Most of the Sr was probably airborne as it was deposited on the leaf surface. Toxic concentrations of

Zn were measured in Belgrade, which might relate to its high mobility and relatively poor sorption in the soil, and pollution resulting from the high traffic flow. Despite the normal concentration of copper in the soil, it is likely that the alkalinity (pH > 8.0), low transfer coefficients and high concentration of Zn in the rooting zone (Zn and Cu compete for the same sites for absorption) are responsible for the lower element solubility and its lower availability for plants. Analysis of trace elements in leaves and bark showed that leaves have higher accumulation capability compared to bark.

Based on RA analysis of the effects of soil chemistry on the accumulation of trace elements in plant tissues it could be concluded that soil chemistry cannot explain the variability of the elements found in the bark (27.6 %), while the percentage of the total variability of elements in the leaves can be explained by the soil chemistry (82.99 %) which suggests that birch leaves are suitable for biomonitoring of pollution from different sources.

The obtained results for photosynthetic pigments revealed a low sensitivity of *B. pendula* to B, Cu, Sr and Zn contamination. It seems that birch, because of the increasing of the amount of pigments, is tolerant to pollution and climate stress and does not suffer damage and metabolic disorder.

SUPPLEMENTARY MATERIAL

Detailed information on study area, species description and sampling are available electronically at the pages of journal website: http://www.shd.org.rs/JSCS/, or from the corresponding author on request.

Acknowledgement. This work was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (Grant No. 173018).

ИЗВОД

МОГУЋНОСТ КОРИШЋЕЊА ЛИСТОВА И КОРЕ *Betula pendula* ROTH. У ПРОЦЕНИ ЗАГАЂЕНОСТИ ЕЛЕМЕНТИМА У ТРАГОВИМА –ИСКУСТВА У СРБИЈИ

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У овом истраживању је проучавана сезонска и просторна динамика акумулације елемената у траговима, као и концентрација фотосинтетичких пигмената у брезама (*Betula pendula* Roth.) урбаних паркова у Панчеву, Смедереву, Обреновцу и Београду (Србија) који су под утицајем различитих антропогених активности. Карактеристике земљишта су одређене у погледу текстуре, pH и садржаја елемената у траговима. Концентрација B, Sr и Zn у оба биљна ткива је показала растући тренд током сезоне, за разлику од Cu који је максималну концентрацију достигао почетком вегетацијске сезоне. Већа акумулација елемената је забележена у листовима у односу на кору са изузетком садржаја Zn. Добијени резултати за фотосинтетичке пигменте су показали слабу осетљивост брезе на загађење B, Cu, Sr и Zn указујући на то да бреза на одабраним локалитетима толерише стресне услове загађења повећавајући количину пигмената. Анализа

ефеката коју садржај елемената у земљишту има на њихову акумулацију је показала да садржај елемената у земљишту слабо потврђује варијабилност елемената у кори (27,6 %) у односу на листове (82,99 %). Линеарна дискриминантна анализа је утврдила да су паркови у Обреновцу и Смедереву јасно одвојени од остала три парка.

(Примљено 13. јануара, ревидирано 14. фебруара, прихваћено 17. фебруара 2017)

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